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Neuroprotective application of *Hemidesmus indicus* root extract and its silver nanoparticles implication against monosodium glutamate-induced neurotoxicity in albino rats

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ABSTRACT

Monosodium glutamate (MSG) induced neurotoxicity is a typical experimental model used to lower memory in laboratory animals. *Hemidesmus indicus* (*H. indicus*) has been used to treat neurotoxicity in Ayurvedic and Unani medicines. The study aimed to assess the neuroprotective efficacy of *H. indicus* root extract and nanoparticles against MSG-induced neurotoxicity in albino rats. The effects of MSG on the brain include neurotoxicity, decreased acetylcholine in rat brains, increased oxidative stress, and increased acetylcholinesterase (AChE) levels after MSG administration. The behavioral, biochemical, and neuroanatomical abnormalities caused by MSG were reduced after treatment with *H. indicus* root extract and its silver nanoparticles (AgNPs) for 21 days. Our study reveals that both the extract and NPs were protecting the rat brain

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against the harmful effects of MSG such as loss of memory and cognitive decline by controlling AChE activity and oxidative stress. AgNPs of *H. indicus* root extract proved extremely significant activity as compared to the extract alone.

Keywords: monosodium glutamate, behavioral impairment, acetylcholinesterase, *Hemidesmus indicus*

INTRODUCTION

Neurotoxicity usually alters the functioning of the central nervous system. It may result in permanent or reversible nerve tissue damage that finally leads to neuronal death.

Grey matter diseases known as neurodegenerative diseases are defined by the progressive death of neurons from particular brain regions. This disorder causes major medical and social issues[1].

Neuroprotection is the body's defense mechanisms and strategies designed to protect the central nervous system from injury caused by both acute and chronic neurological conditions, such as dementia, Parkinson's disease, Alzheimer's disease and epilepsy. Dementia, also known as Alzheimer's disease, is a long-term cognitive and emotional condition that affects a person's ability to function normally[2]. The prevalent type of dementia is Alzheimer's disease, which is characterized as a neurotoxin disorder that most frequently causes memory loss and cognitive deterioration[3]. Alzheimer's disease affects an estimated 50 million people globally and is projected to affect 152 million people by 2050[4]. Monosodium glutamate (MSG) is one of the most commonly used flavor enhancers and a controversial food additive, found in almost all types of fast foods, packed Chinese food, soups, canned vegetables and processed meats. MSG is sodium salt of a naturally occurring non-essential amino acid L glutamic acid and is the most abundant amino acid found in nature[5]. Monosodium glutamate administration led to acute increase in intracerebroventricular and hippocampal glutamate concentrations. Glutamate, which is released from about 40% of synapses in the central nervous system, is the most prevalent excitatory neurotransmitter in the brain. Along with playing a crucial role as a neurotransmitter, glutamate may also act as a powerful neurotoxin to neurons when levels are too high. It is still unknown how cells die, despite the fact that glutamate-induced cell death is linked to both apoptotic and necrotic changes. The excitotoxic pathway and the oxidative pathway are two distinct pathways for glutamate-induced cell death that have each been described. The excito-

toxic pathways involve excessive glutamate receptor activation, which causes both quickly triggered and gradually triggered cytotoxic events[6]. The extract and nanoparticles of *H. indicus* prevents monosodium glutamate induced neurotoxicity due to its anti-oxidative property. The neuroprotective activity of *H. indicus* might be due to the presence of various phytochemical constituents such as β-sitosterol, tannins, saponins, flavonoids, alkaloids and higher content of phenols and free amino acids[7].

The multidisciplinary nature of nanotechnology makes it one of the fastest-growing fields of science[8].As AgNPs have attracted significant attention because of their unique properties and potential uses in a wide spectrum of application and various features such as stable morphology, less particle size, high surface-to-volume ratio, high bioavailability and useful chemical properties relating to surface and cell penetration capability which can be useful for many purposes[9]. AgNPs are found to be metal nanoparticles with a large absorption band[10]. Due to their specific physical and chemical characteristics, AgNPs are used in many kinds of industrial and medicinal applications. AgNPs have attractive physicochemical properties, low toxicities, the ability to generate a wide range of nano structure, and low manufacturing costs. Additionally, AgNPs have electronegativity, optical properties, and biological properties[11,12]. Indian Sarsaparilla, also known as *H. indicus* (Asclepiadaceae), is a popular plant that may be found in India. It is frequently utilised in Indian traditional medicine, and its medicinal uses have been studied extensively[13]. *H. indicus* is widely used as a traditional folk remedy and is also an ingredient of Ayurveda and Unani medicinal products aganist various diseases such as rheumatism, kidney and urinary disorders, diarrhoea, skin issues and asthma. It has also been reported for its neuroprotective activity[14]. A systematic literature survey has not yielded scientific evidence to prove the neuroprotective activity of AgNPs of *H. indicus* root. So, the present study aims to evaluate the neuroprotective efficacy of *H. indicus* root extract and its AgNPs in MSG-induced neurotoxicity in albino rats.

METHODOLOGY

Plant material

The roots of *H. indicus* were collected From the Bellary district. The roots were authenticated by Dr. Pradeep, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara college of Ayurveda and Hospital, Hassan, Karnataka, India (Voucher Number of the plant: SDMCAH-DG/2023/03). The collected roots were washed, dried, subjected for size reduction into a coarse powder and stored in an airtight container at room temperature for further usage.

Preparation of extract

The roots of *H. indicus* were extracted by cold maceration method using 70% ethanol. Roots residue was removed from the extract using Whatman No. 1 filter paper. The obtained clear extract was stored in a refrigerator for further investigation[15].

Preparation of nanoparticles

A 250 mL volumetric flask contains 180mL of silver nitrate solution (1mM) and 20mL of *H. indicus* root extract was swirled twice for 5min at room temperature using a magnetic stirrer. After one week, the colour change indicates the presences of AgNPs. Then, a UV-spectrophotometer was used to assess absorbance (200 to 700 nm). After the reaction was finished, the solution was centrifuged at 5000rpm for 15min at 40°C. The pellet that had collected in the tubes bottoms was removed and dried for 5hrs in a hot air oven at 80°C[16].

Zeta potential

A zeta potential study was conducted to determine the surface charge of the prepared material and its stability *H. indicus* AgNPs. Method used to determine the Zeta potential is dynamic light scattering (DLS) with Malvern-Model Zeta sizer Nano ZSP which is used to control the stability of the sample[17].

Fourier Transform Infrared Spectroscopy (FTIR)

In order to identify the sample functional groups, we used the (Shimadzu 8400S, Shimadzu, Kyoto Japan) FTIR, and the FTIR measurements were made to determine the silver ions and compounds responsible for reducing silver nitrate to AgNPs. The sample was then placed on ATR and analyzed using FT-IR[18].

Scanning Electron Microscopy (SEM) analysis

SEM was utilized to determine the surface structure of the bio-generated AgNPs (Hitachi s3400n, Japan)[10].

Experimental animals

This study involved female Wistar rats that weighed between 150-180 gms. The rats were taken from an approved breeder and kept at a temperature of 24 to 28°C. The cages were sterilized, and the animals were kept for 21 days. They were given a regular pellet diet with water *adlibtum*[19]. The Institutional Animal Ethics Committee approved the study, which was done at the Sri Adichunchanagiri college of Pharmacy in Karnataka, India (Approval No: SACCP-IAEC/2022-02/66).

Experimental design

Fourth two animals were divided into seven groups with six in each group.

Group 1- Normal control (received vehicle)

Group 2- Received MSG (2g/kg) from 8th to 21days with no extract

Group 3- Received Piracetam (140mg/kg) + MSG (2g\kg) for 21 days

Group 4- Received *H. indicus* root extract for 21 days (300mg\kg) + MSG (2 g\kg) from 8th to 21st day

Group 5- *H. indicus* root extract for 21 days (150mg\kg) +MSG (2 g\kg) from 8th to 21st day

Group 6- AgNPs of *H. indicus* root extract for 21 days (50mg\kg) + MSG (2 g\kg) from 8th to 21st day

Group 7- AgNPs of *H. indicus* root extract for 21 days (100mg\kg) + MSG (2 g\kg) from 8th to 21st day

Morris Water Maze (MWM) (San Diego Instruments, United States)

Before the research began, all laboratory animals were given 3days of training. Within the study target quadrant (Q4) of this pool, a submerged platform was positioned 1cm below the water surface. Each rat was dropped into the water in a different spot between the quadrants, facing the wall of the pool. They were then given 120sec to find the submerged platform. The platform location remained stable during the event. For 4days (days 5, 8 and 14) each animal had two consecutive trials with a 5min gap between them. During these trials, the animal was allowed to run onto the hidden platform and remain there for 20sec. On the 21st day, the platform was removed and rats were assigned a position in any one of the three quadrants (Q1, Q2, or Q3) and given 120 sec to explore the target quadrant. As a measure of memory, the typical amount of time spent in the target quadrant (Q4) in search of the missing platform was recorded[20].

Novel object recognition

The experimental set-up for the object recognition task includes an open field box made of black acrylic that is 40 by 40 by 40 cm in size. The behavior test was conducted under low red-light illumination. Before the initiation of the project, all laboratory animals underwent training, each with a different starting position for 1week. MSG was given to lab animals on day 8 for 21 days, and then *H. indicus* was given on day 1 to 21 days. The things to be distinguished

were a toy Lego set, a novel object and two identical clear cultured flasks with water. The day before the trial, each rat got habituated to the open field box for 10min without any objects. On the day of the experiment, each rat was allowed to investigate two identical objects (transparent cultured flasks filled with water) for 5min in the 1st trial. One of the training session old objects was swapped out for a new one, and the rat was given 2min to investigate the new ones. The time spent near each object was recorded. When they were shown during the test session, the two items had different textures, colors, and sizes. Between runs, the open field box was cleaned with 70% ethanol to reduce fragrance remnants. The formula [TB / (TA + TB)*100] was used to determine the recognition index, where TA and TB stand for the length of time spent examining known object A and novel object B respectively. A rat was considered to have explored an object when it touched it with its forepaws or nose[21].

Preparation of brain supernatant

On day 22, animals were sacrificed by cervical dislocation and the brain was isolated. The brain was cleaned with 0.9% saline and stored on ice. The brain was then blotted with filter paper. The brain and hippocampus were extracted, and the cerebrum was homogenized (20% w/v) in cold phosphate buffer. The homogenate (pH 8 = 0.1M) was centrifuged at 3000rpm for 10min at 4°C. The cloudy supernatant fluid was used to calculate brain acetylcholinesterase activity[22].

Estimation of acetyl cholinesterase (AChE) activity

The Ellman method was used to evaluate the AChE activity. The test sample contains 0.05mL of supernatant. 3mL of sodium phosphate buffer Acetylthiocholine iodine 0.1 mL of acetylthioquinolone DTNB 1mL of Ellman reagent. Perkin Elmer lambda 20 spectrophotometer was used to measure the change in absorbance at 412nm for 2min at 30sec intervals[23].

Histopathological analysis

For histopathological studies, the brain was separated and preserved in a 10% formalin solution[24].

Statistical analysis

The data were presented as Mean ± SEM and statistically significant ($p < 0.001$). ANOVA (analyses of variance) was used to compare groups and Tukey's test was used to determine *p*-values.

RESULTS and DISCUSSION

Around 50 million people worldwide suffer from Alzheimer's disease and by 2050, the total population is projected to triple every five years to reach 152 million[4]. Monosodium glutamate produces a specific taste called umami through activation of the TAS1R1-TAS1R3 in the tongue. Due to its unique taste, the use of MSG is increased worldwide. Monosodium glutamate is problematical so it should be used with limitations according to the permissible amounts stated by different organizations to avoid its adverse effects[25]. The presence glutamic acid is responsible for triggering neuronal migration and differentiation, synaps remodeling and long-term potentiating, yet excessive concentration of this compound may cause neuronal death. In the recovery trial of the Morris water maze test, the MSG-induced rat took longer to find the hidden platform, which is a sign of memory impairment, according to our findings. The Novel objective recognition test suggested that the Piracetam, *H. indicus* root extract, nanoparticles group rats significantly reduced the amount of time spent in the novel object when compared to the MSG-treated group. Levels of acetylcholinesterase in *H. indicus* demonstrated that MSG administration substantially increased the activity of acetylcholinesterase in rats compared to the control group. Additional evidence came from the abnormal brain structure, which also shows that MSG has caused memory impairment and the involvement of certain brain regions in cognitive functions. In Treatment with *H. indicus* extract, nano-particles resulted in an increase in pyramidal cells and a decrease in cytoplasm vacuolation. Our results showed that *H. indicus* extract and its nano-particles, treated the memory impairment caused by MSG, while maintaining brain structure and preventing neurodegeneration, by reducing brain AChE activity and oxidative stress. Histological analysis of the brains of the MSG-treated rats revealed observable vacuolar changes, oedema and modest signs of inflammation in cortical regions in comparison with the control group. When compared to *H. indicus* extract and silver nanoparticles, the silver nanoparticles have higher levels of neuroprotective action.

Fourier Transfer Infra-Red rays

The FTIR spectrum was observed within a range of 4000-500 cm^{1} and it indicates that the *H. indicus* AgNPs contains numerous functional groups that serve as reducing and stabilizing agents before the synthesis of NPs. The different peaks of *H. indicus* are represented in Figure 1 and Table 1.

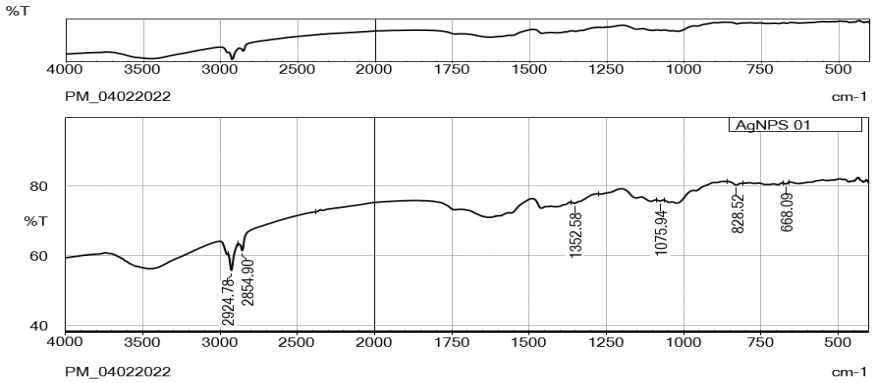

Figure 1. Structural features of the *H. indicus*AgNPs by FTIR spectrum

Table 1. Wave number and functional groups of *H. indicus*AgNPs

Wave numbers (cm-1)	Assignments
2924.78	OH(Broad)
2854.90	C6H6O
1351.16	C≡C (week)
1075.61	Mononuclear aromatics
828.52	Oxirane ring
668.09	Benzene (Ring bending bands)

Zeta potential

The surface charge of the produced NPs and the stability of the synthesized NPs were determined in a zetapotential investigation. A clear signal was observed at -8mv, which can be observed in Figure 2, suggesting that the synthesized NPs had good stability. This indicates that intermediate stability is attributed to the presence of capping molecules on the surfaces of biosynthesis-based Ag-NPs, which are predominantly composed of negatively-charged groups (Table 2). It is speculated that the incorporation of components such as proteins or flavonoids into AgNPs is responsible for the reduction in metal ions and the successful stabilization of the AgNPs.

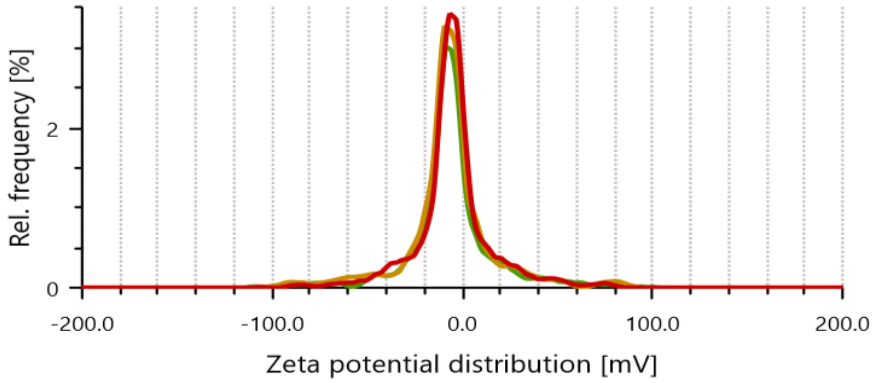

Figure 2. Zeta potential of the *H. indicus*AgNPs

Table 2. Mean zeta potential, conductivity, adjusted voltage and processed runs

Sl.No	Mean zeta pot. [mV]	Electroph. Mobil. [µm*cm/ Vs]	Conductivity [mS/cm]	Adjusted voltage [V]	Processed runs
1	-8.0	-0.4137	3.707	10.0	140
2	-8.3	-0.4332	3.704	10.0	100
3	-7.7	-0.4015	3.706	10.0	120

Scanning electron microscopy

The SEM analysis of the AgNPs from *H. indicus* showed morphological homogeneity in the AgNPs distribution on the grid surface. Although spherical NPs of various sizes tend to prevail, SEM displays an abundance of NPs with a range of shapes. The synthesized AgNPs were found to have an average size of 73.2 nm as shown in Figure 3.

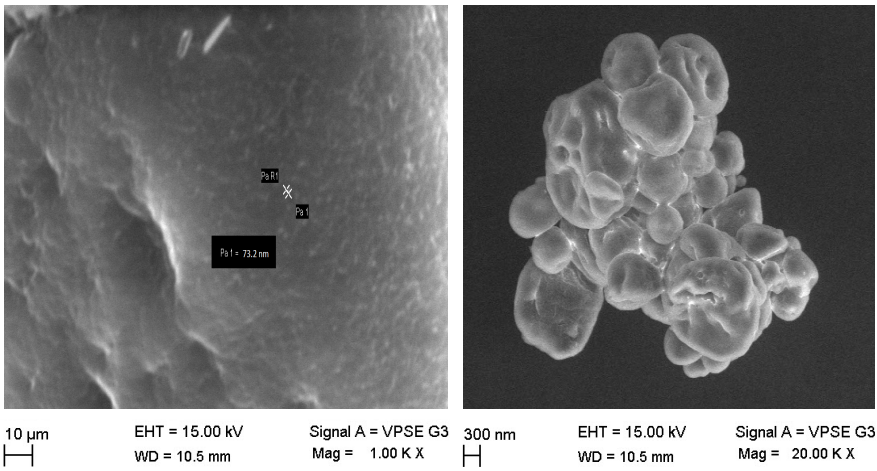

Figure 3. SEM image shows the shape of variable size of AgNPs

Morris Water Maze

The acquisition latency for reaching the visual platform was considerably delayed in MSG-treated rats is compared to the control group, demonstrating memory impairments. On days 5, 8 and 14, the *H. indicus* extract (150 and 300mg/kg) and its AgNPs (50 and 100mg/kg) treatment enhanced memory function (reduced mean acquisition latency) in the MSG-treated group significantly. After training, the visible platform was maintained 1cm below the waterline. In comparison with the control group, the MSG treatment significantly increased the mean acquisition latency and retention latency (days 5, 8 and 14 respectively) to reach the hidden platform which has been depicted in Figure 4 and Figure 5. These findings imply that MSG significantly impaired cognitive function. Furthermore, compared to animals treated with MSG, *H. indicus* roots extract and AgNPs administration significantly boosted memory recall on days 5, 8 and 14 respectively. On the 21st daytime spent in the target quadrant by the NPs-treated group showed highly significant activity as compared with inducer alone. The activity is highly significant in *H. indicus* AgNPs as compared to *H. indicus* extract alone. The data is given in Table 3 and Table 4.

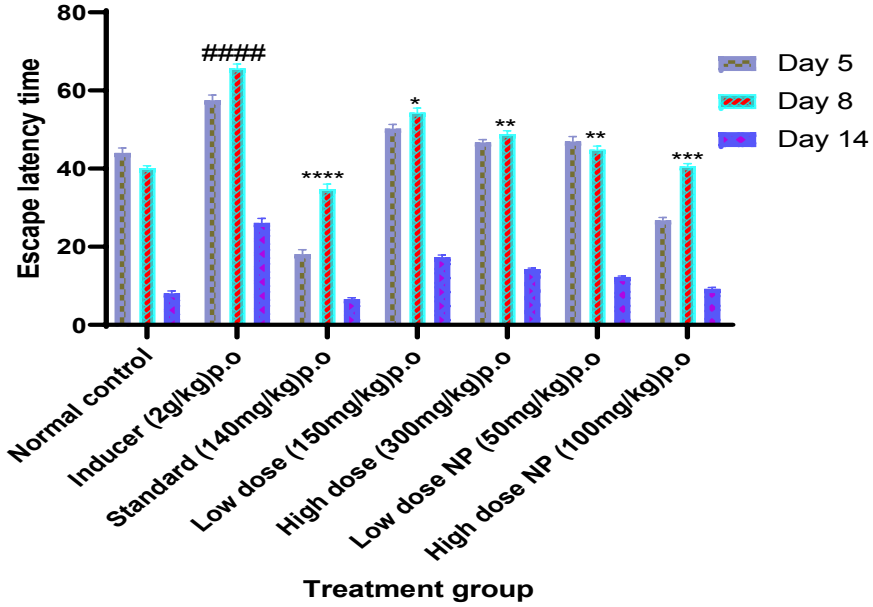

Figure 4. Effect of *H. indicus* on escape latency time (ELT) of rats by using Morris Water Maze on the 5, 8 and 14 days

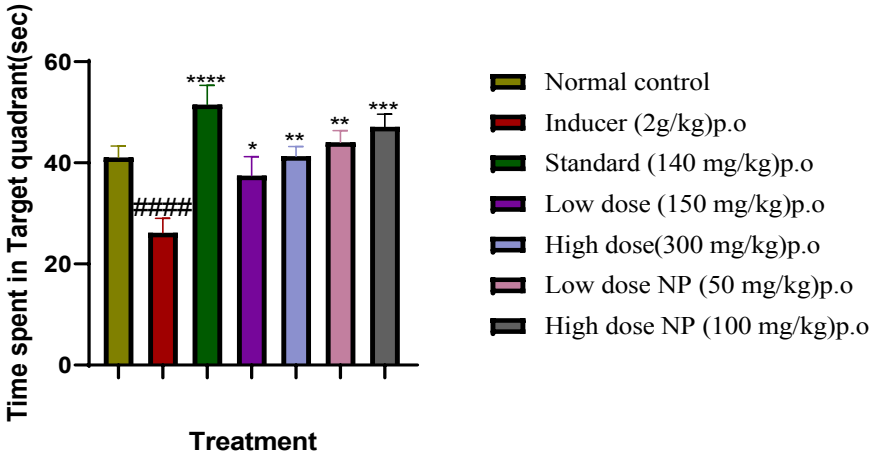

Figure 5. The effect of *H. indicus* on time spent in the target quadrant of rats by using MWM on 21th day

Table 3. Effect of *H. indicus* on escape latency time (ELT) of rats by using Morris Water Maze on the 5, 8, 14 days

Group No	Treatment	ELT (sec) Mean±S.E.M [n=6]		
		On 5th day	On 8th day	On 14th day
1	Control	44.0 ± 1.309	40.13 ± 0.6331	8.117 ± 0.5755
2	Monosodium glutamate (2g/kg)	57.52 ± 1.295	65.68 ± 1.093	26.13 ± 1.152
3	Piracetam (140mg/kg) + MSG (2g/Kg)	18.10 ± 1.179****	34.77 ± 1.279****	6.583 ± 0.3635****
4	Plant extract treated (150 mg/kg) + MSG (2g/Kg)	50.23 ± 1.099*	54.37 ± 1.144 *	17.37 ± 0.4888*
5	Plant extract treated (300 mg/kg) + MSG (2g/Kg)	46.70 ± 0.7131**	48.87± 0.8582**	14.28 ± 0.2354**
6	AgNPs treated (50mg/Kg) + MSG (2g/Kg)	46.93 ± 1.262**	44.87± 0.8982**	12.28 ± 0.2354**
7	AgNPs treated (100mg/Kg) + MSG (2g/Kg)	26.77 ± 0.6965***	40.60 ± 0.6814***	9.250 ± 0.3293***

The behavioral analysis was compared to an inducer control group (monosodium glutamate). The data are presented as Mean ± SEM, n = 6, and statistical analysis is conducted using a Two-way (ANOVA) followed by a Tukey's test. *p<0.05, **p<0.01 ***p<0.001compared to an inducer control group (monosodium glutamate).

Table 4. Effect of *H. indicus* on time spent in the target quadrant of rats by using Morris Water Maze on the 21st day

Group No	Treatment	TSTQ Mean±S.E.M [n=6]
1	Control	41.05 ± 0.8660
2	Monosodium glutamate (2g/kg)	26.18 ± 1.066
3	Piracetam (140mg/kg) + MSG (2g/kg)	51.50 ± 1.443****
4	Plant extract treated (150mg/kg) +MSG (2g/kg)	37.48 ± 1.412*
5	Plant extract treated (300mg/kg) + MSG (2g/kg)	41.33 ± 0.7127**
6	AgNPs treated (50mg/kg) + MSG (2g/kg)	44.07 ± 0.8706**
7	AgNPs treated (100mg/kg) + MSG (2g/kg)	47.13 ± 0.9349***

The behavioural analysis was compared to an inducer control group. The data are presented as Mean ± SEM, n = 6, and statistical analysis is conducted using a Two-way (ANOVA) followed by a Tukey's test. *p<0.05, **p<0.01 ***p<0.001compared to an inducer control group (monosodium glutamate).

Novel object recognition

Piracetam, *H. indicus* extract and *H. indicus* AgNPs significantly decreased the time spent by the rat near the novel object when compared to the MSG-treated group, as depicted in Figure 6. Rat's acceptance of novelty is usually measured using the discrimination index, as depicted in Figure 7. The MSG-treated group of rats showed poor object recognition as compared to the normal control group. MSG-induced rats treated with piracetam, *H. indicus* extract and its AgNPs spent more time near the novel object. Treatment also increased the object recognition ability in rats. *H. indicus*AgNPs possess highly marked activity as compared to extract alone. The data is shown in Table 5 and Table 6.

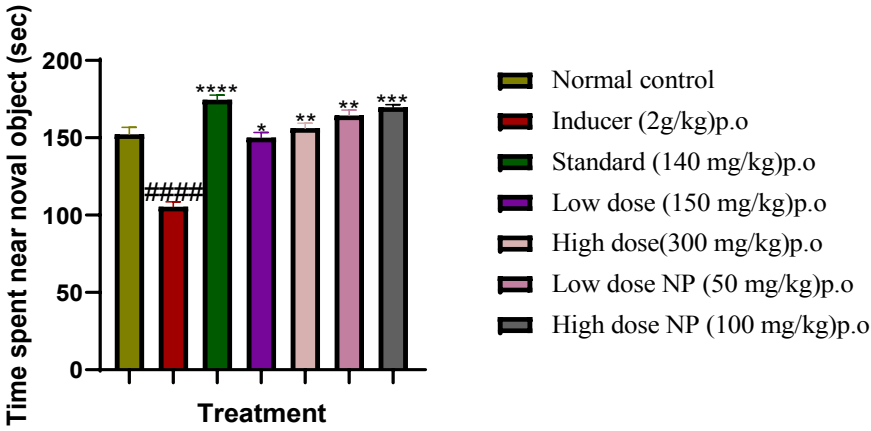

Figure 6. Effect of *H. indicus* on Time spent near novel object of rats by using NOR test

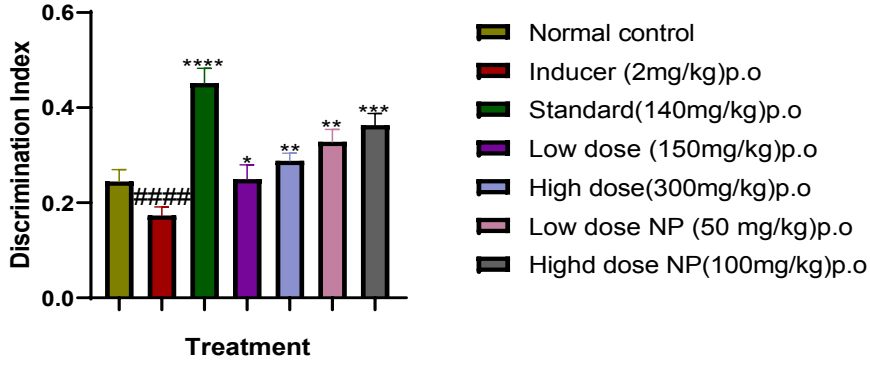

Figure 7. Effect of *H. indicus* on Discrimination index of rats by using NOR test

Table 5. Effect of *H. indicus* on time spent near novel object of rats by using Novel Objective Recognition test

Group No	Treatment	Time spent near novel object Mean±S.E.M [n=6]
1	Control	152.2 ± 1.751
2	Monosodium glutamate (2g/kg)	105.4 ± 1.218
3	Piracetam (140mg/kg) + MSG (2g/kg)	174.6 ± 1.132****
4	Plant extract treated (150mg/kg) +MSG (2g/kg)	150.1 ± 1.235*
5	Plant extract treated (300mg/kg) + MSG (2g/kg)	156.3 ± 1.159**
6	AgNPs treated (50mg/kg) + MSG (2g/kg)	164.4 ± 1333**
7	AgNPs treated (100mg/kg) + MSG (2g/kg)	169.8 ± 0.5378****

The behavioral analysis was compared to an inducer control group (monosodium glutamate). The data are presented as Mean ± SEM, n = 6, and statistical analysis is conducted using a Two-way (ANOVA) followed by a Tukey's test. *p<0.05, **p<0.01 ***p<0.001 compared to an inducer control group (monosodium glutamate).

Table 6. Effect of *H. indicus* on discrimination index of rats by using Novel Objective Recognition test

Group No	Treatment	Discrimination (DI) Mean ± S.E.M [n=6]
1	Control	0.2450 ±0.0094
2	Monosodium glutamate (2g/kg)	0.1733 ±0.0067
3	Piracetam (140mg/kg) + MSG (2g/kg)	0.4517 ±0.01184****
4	Plant extract treated (150mg/kg) +MSG (2g/kg)	0.2500 ±0.0059*
5	Plant extract treated (300mg/kg) + MSG (2g/kg)	0.2883 ±0.00594**
6	AgNPs treated (50mg/kg) + MSG (2g/kg)	0.3283 ±0.00986**
7	AgNPs treated (100mg/kg) + MSG (2g/kg)	0.3633 ±0.009172***

The behavioral analysis was compared to an inducer control group (monosodium glutamate). The data are presented as Mean ± SEM, n = 6, and statistical analysis is conducted using a Two-way (ANOVA) followed by a Tukey's test. *p<0.05, **p<0.01 ***p<0.001compared to an inducer control group (monosodium glutamate).

Acetylcholine assays

The Acetylcholine esterase activity was greatly increased in MSG treated rats as compared to normal control rats. In comparison to MSG induced rats, *H. indicus* root extract and its nanoparticles therapy continuously reduced acetylcholine esterase activity as shown in Figure 8 and Table 7.

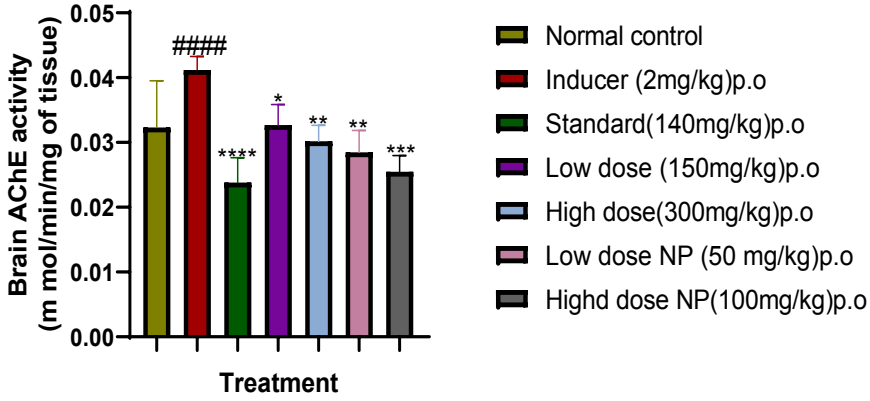

Figure 8. Effect of HI on AChE on rat brain

Table 7. Effect of *H. indicus* on AChE of rats

Group No.	Treatment	AChE Mean ± S.E.M [n=6]
1	Control	0.0323 ± 0.00272
2	Monosodium glutamate (2g/kg)	0.04117 ± 0.00079
3	Piracetam (140mg/kg) + MSG (2g/kg)	0.02383 ± 0.00143****
4	Plant extract treated (300mg/kg) +MSG (2g/kg)	0.03267 ± 0.001208*
5	Plant extract treated (150mg/kg) + MSG (2g/kg)	0.03017 ± 0.0009365**
6	AgNPs treated (100mg/kg) + MSG (2g/kg)	0.02850 ± 0.001268**
7	AgNPs treated (50mg/kg) + MSG (2g/kg)	0.0255 ± 0.0009449***

The behavioral analysis was compared to an inducer control group (monosodium glutamate). The data are presented as Mean ± SEM, n = 6, and statistical analysis is conducted using a Two-way (ANOVA) followed by a Tukey's test. *p<0.05, **p<0.01 ***p<0.001compared to inducer control (monosodium glutamate).

Histopathological examination (Brain cell)

- Normal Control-there are no intracellular spaces, cells are linearly arranged, no shrunken cells and well-organized pyramidal cells were observed (Figure 9-A).
- Inducer (MSG)-there are more intracellular spaces, cells are not linearly arranged, pyramidal cells are unorganized, and cells are more shrunken (Figure 9-B).

- Standard (piracetam)-no intracellular spaces, cells are linearly arranged, no shrunken cells observed, pyramidal cells are well organized (Figure 9-C).
- *H. indicus* extract low dose-there are adequate intercellular spaces were observed and cells are well organized, less shrunken cells (Figure 9-D).
- *H. indicus* extract High dose-there are adequate intercellular spaces were observed and cells are well organized, less shrunken cells (Figure 9-E).
- *H. indicus*AgNPs low dose-there are adequate intercellular spaces were observed and cells are well organized, less shrunken cells (Figure 9-F).
- *H. indicus*AgNPs low dose-adequate intercellular spaces were observed and cells are well organized, less shrunken cells, and cell morphology changes are not seen (Figure 9-G).

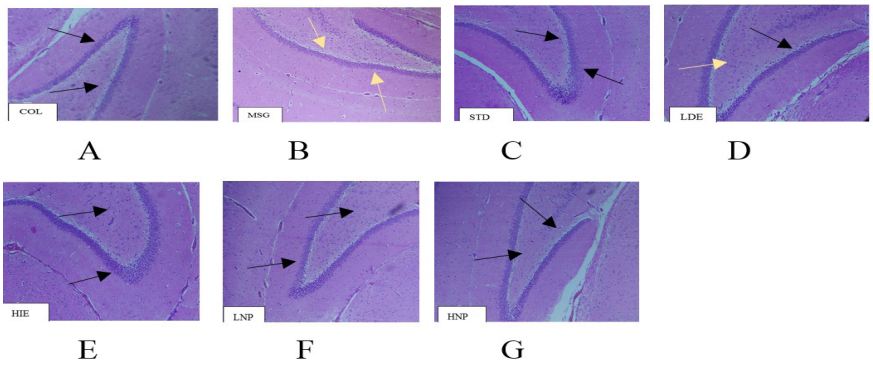

Figure 9. Histopathological Observation, (A):normal control, (B):Monosodium glutamate (inducer 2g/kg(p.o)), (C):Piracetam (standard 140mg/kg(p.o)), (D):Low Dose 150mg/kg(p.o), (E):High dose 300mg/kg(p.o), (F):Low dose nanoparticle 50mg/kg(p.o), (G):High dose Nanoparticle 100mg/kg(p.o).

Black- Normal pyramidal cells observed, less intercellular spaces and the cells are linearly arranged, no apoptosis is observed. Yellow- Pyramidal cells are shrunken, more intercellular spaces are observed, and cells morphology changes are shown.

This study shows that MSG treatment in rats causes behavioral and cognitive abnormalities as well as histological and biochemical alterations in the brain. Our results showed that therapy with *H. indicus* roots extract and nano-particles lowers MSG-induced behavioral and cognitive impairment and reduces neurotoxicity. *H. indicus* root extract and nano-particles also help to decrease the effects of MSG-induced oxidative stress and AChE activity in the brain. These results suggest that *H. indicus* enhances cholinergic transmission and

decreases oxidative stress to protect against neurodegeneration in the brain and maintain memory and cognitive functioning. The neuroprotective potential of *H. indicus* silver nanoparticles were higher than the extract alone. Further study is required to exactly understand how the phytoconstituents of *H. indicus* roots interact with biochemical pathways in the brain to the presence of its neuroprotective activity.

STATEMENT OF ETHICS

The animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) at Sri Adichunchanagiri College of Pharmacy, B.G Nagara, under the approval number SACCP-IAEC/2022-02/66. The procedures adhered to the guidelines set forth by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), India.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

MC: Design, acquisition of data, analysis of data, drafting of manuscript, statistical analysis. PRC: Design, acquisition of data. SSA: Design, critical review of manuscript, supervision. ABV: Design, plant collection and authentication. BDR, BR: Design, critical review of manuscript, supervision.

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